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Research report

Glucagon-like peptide-2 but not imipramine exhibits antidepressant-like effects in ACTH-treated mice

Takashi Iwai[a,b], Tomoko Ohnuki[a], Sachie Sasaki-Hamada[a], Akiyoshi Saitoh[a,b], Azusa Sugiyama[a,b], Jun-Ichiro Oka[a,*]

[a] Laboratory of Pharmacology, Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba, 278-8510, Japan
[b] Department of Neuropsychopharmacology, National Institute of Mental Health, National Center of Neurology and Psychiatry, Tokyo 187-8553, Japan

HIGHLIGHTS

- We investigated the effectiveness of GLP-2 in ACTH-treated mice using FST.
- GLP-2 but not imipramine induced antidepressant-like effects.
- GLP-2 attenuated the increase of serum corticosterone levels caused by the FST.
- GLP-2 changed 5-HT and 5-HIAA levels.
- GLP-2 induced antidepressant-like effects under imipramine-resistant conditions.

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ABSTRACT

We investigated the effectiveness of glucagon-like peptide-2 (GLP-2) against refractory depression in adrenocorticotropic hormone (ACTH)-treated mice as a model of tricyclic antidepressant (TCA)-resistant depression. Chronic ACTH treatment (0.45 mg/kg, s.c., 14 days) weakened the antidepressant-like effects of imipramine (20 mg/kg, i.p., 6 days) in the forced-swim test (FST). Conversely, GLP-2 (3 μg/mice, i.c.v., 6 days) induced antidepressant-like effects in the ACTH-treated mice in the FST. ACTH-treatment increased basal serum corticosterone levels, with an additional increase induced by the FST. Imipramine or GLP-2 had no effect on the basal corticosterone level, but GLP-2 attenuated the additional increase caused by the FST. Moreover, GLP-2 increased 5-HT levels, but not 5-HIAA. These results suggest that GLP-2 induced antidepressant-like effects under imipramine-resistant conditions through increase in 5-HT levels.

1. Introduction

Glucagon-like peptide-2 (GLP-2) is derived from a proglucagon precursor and liberated via tissue-specific post-translational processing in the gut and central nervous system [18]. GLP-2 was identified as a potent intestinotrophic hormone in rodents [3], and enhanced nutrient absorption in rodents and in patients with short bowel syndrome [9]. On the other hand, intracerebroventricular (i.c.v) injection of GLP-2 inhibited food intake in rats [23]. In addition to its anorexigenic effects, GLP-2 protected hippocampal neurons from glutamate excitotoxicity [17], stimulated the proliferation of cultured cortical astrocytes of rats [24–26], and enhanced L-type calcium ion channel activity [28].

We previously reported that GLP-2 has antidepressant-like effects in the forced-swim test (FST) and the tail suspension test [7]. Major depression is a psychiatric disease that results in dramatic alterations in emotional, neurovegetative, and cognitive processes. Although antidepressants are clearly beneficial for the treatment of major depression, their use presents many problems such as side-effects, relapse, refractory patients, and delayed onset of action. Consequently, there remains a pressing need for new antidepressant drugs.

It was previously reported that rats repeatedly treated with adrenocorticotropic hormone (ACTH) served as an animal model of tricyclic antidepressant-resistant depression with a predictive validity [10,11]. Neither imipramine nor desipramine had any effect on immobility time in the FST among the ACTH-treated rats, the

Abbreviations: ACTH, Adrenocorticotropic hormone; FST, Forced-swim test; 5-HT, Serotonin; 5-HIAA, 5-Hydroxyindoleacetic acid; HPA, Hypothalamic–adrenal axis; GLP-2, Glucagon-like peptide-2; PBS, Phosphate-buffered saline.

* Corresponding author. Tel.: +81 4 7121 3610; fax: +81 4 7121 3610.
E-mail address: okaji@rs.noda.tus.ac.jp (J.-I. Oka).

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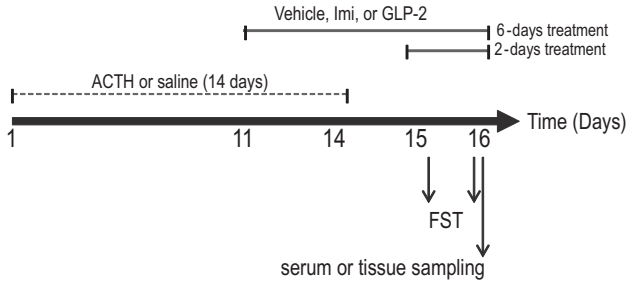

Fig. 1. Experimental schedule. Solid lines indicate drug (Imi: 30 mg/kg, i.p.; GLP-2, 1.5–6 μg/mice, i.c.v.) or vehicle (saline, i.p. or PBS, i.c.v.) treatment for 2 (Day −15, −16) or 6 (Day −11 to −16) days. Dashed lines indicate chronic treatment with ACTH (0.45 mg/kg, s.c.) or vehicle for 14 days (Day −1 to −14). The FST was performed at Day −15 (training session) and −16 (test session). Serum or brain tissue samples were collected at Day −16. Imi: imipramine.

stress causing an additional increase in serum corticosterone levels. The use of lithium together with tricyclic antidepressants or electroconvulsive treatment resulted in antidepressant-like effects in ACTH-treated rats [10,13]. Although adjunct lithium is in reality off-label in the US (atypical antipsychotics are approved), these were considered the effective treatment for antidepressant-resistant depression [2,14]. We treated mice with ACTH according to the method described by Kitamura et al. [10] with some modifications, and observed imipramine-resistant properties in the FST similar to the ACTH-treated rats (Fig. 1). We hence used ACTH-treated mice to model tricyclic antidepressant-resistant depression and investigated the potential ability of GLP-2 to treat refractory depression.

Next, we measured serum corticosterone, serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of 5-HT, to support the behavioral effects of GLP-2. Higher corticosterone levels indicate a dysfunctional hypothalamic-adrenal axis (HPA) in response to stress, and were found in patients with major depression [1]. In addition, increasing extracellular 5-HT is the action of most antidepressants widely used.

2. Materials and methods

All experimental protocols were approved by the Institutional Animal Care and Use Committee at Tokyo University of Science, and were conducted according to the guidelines of the National Institutes of Health and the Japan Neuroscience Society.

2.1. Animals

Six-week-old male ddY mice (Japan SLC, Shizuoka, Japan) were kept in a controlled environment, with controlled lighting (12 h light/dark cycle, lights on from 08:00 to 20:00), temperature (23 ± 1 °C), and relative humidity (55 ± 5%) for at least 5 days before the experiments, and were given free access to food and water.

2.2. Drug treatment

The experimental schedule is indicated in Fig. 1. ACTH (Cortrosyn-Z: Daiichi Seiyaku, Tokyo, Japan) was diluted with saline. ACTH (0.45 mg/kg, s.c.) or saline (vehicle control) was administered once a day for 14 days at 09:00–11:30. Imipramine (Sigma) was dissolved with saline, and administered for 2 (Day − 15, 16) or 6 (Day −11 to −16) days (30 mg/kg, i.p.). GLP-2 (Peptide inc., Osaka, Japan) was dissolved in 0.01 M PBS. GLP-2 or PBS (vehicle control) was administered into the lateral ventricular (i.c.v.) region of the mouse brain once a day for 2 (Day −15, 16) or 6 (Day −11 to −16) days, since there is no report about the ability of GLP-2 to penetrate the blood-brain barrier. The i.c.v. administration (a volume of 5 μl/mouse) was performed under brief ether anesthesia according to the method of Haley and McCormick [4]. On the day of the FST session, imipramine, GLP-2 or vehicle (saline or PBS) was administered 30 min before the test.

2.3. Forced-swim test and Drug treatment

The FST was performed as described previously [7]. The test was performed by placing a mouse in an acrylic cylinder (50 cm tall, 18 cm in diameter) containing a 7-cm water column (25 °C). The water was replaced between every trial. Two swimming sessions were conducted at Day −15 and −16 (Fig. 1): an initial 15-min pretest, followed by a 5-min test 24 h later. Test sessions were video-taped to measure the time of immobility, and immobility was defined as floating passively in the water and only making slight movements to keep the head above the water line. The scored immobility time was blindly checked by co-authors.

2.4. ELISA

Blood samples were collected 30 min after the final treatment or immediately after the FST (Day −16, Fig. 1). The samples were centrifuged for 15 min (×1000 g) at 4 °C, and the serum fraction was collected. Serum corticosterone concentrations were measured using a commercially available ELISA kit (corticosterone EIA Kit, Cayman Chemical Company).

2.5. Analysis of the concentration of 5-HT and its metabolite with HPLC

The concentrations of 5-HIAA were determined by high-performance liquid chromatography (HPLC). Thirty minutes after the last administration (Day −16, Fig. 1), the animals performed the FST. Immediately after the FST, all mice were killed by decapitation. The frontal cortex and hippocampus were quickly dissected. These brain tissues were stored at −80 °C until further use. The tissues were homogenized in 300 μL of 0.2 M perchloric acid containing 100 μM EDTA (2 Na) and 100 ng of isoproterenol as an internal standard. To remove proteins completely, the homogenates were placed in cold water for 30 min and then centrifuged at 14,500× g for 15 min at 0 °C; the upper layer was maintained at pH 3.0 using 1 M sodium acetate. Samples of 20 μL were analyzed by HPLC using electrochemical detection. The electrochemical detector (ECD-300, Eicom Co., Kyoto, Japan) was equipped with a graphite electrode (WE-3G, Eicom Co., Kyoto, Japan) that was used at a voltage setting of 750 mV vs. an Ag/AgCl reference electrode. The mobile phase consisted of a 0.1 M sodium acetate/0.1 M citric acid buffer (pH 3.5) containing 17% methanol, 210 mg/L sodium 1-octanesulfonate, and 5 mg/L EDTA (2 Na). The monoamines were separated on a C-18 column (150 mm × 3.0 mm reversed-phase, EICOMPAK SC- 5ODS, Eicom Co.). The mobile phase flow rate was maintained at 0.5 mL/min with a column temperature of 25 °C.

2.6. Data analysis

The data are expressed as the mean ± S.E.M. The significance of differences was evaluated using Student's *t*-test (for 2 groups) or a parametric one-way analysis of variance (ANOVA) followed by Dunnett's test or Bonferroni's test. If the variances of each group were unequal, a non-parametric Mann–Whitney's test was used for the comparison of 2 groups. All statistical analyses were performed using Graphpad Prism version 4 or 5 (Graphpad Software Inc., San Diego, CA, USA.). The criterion for significance was $P < 0.05$ in all statistical evaluations.

3. Results

3.1. Effects of imipramine on immobility time in the FST

To investigate whether ACTH-treatment weakened the effects of imiplamine, we performed the FST after saline or imiplamine treatment (30 mg/kg, i.p., 6 days). Imipramine significantly decreased immobility time in the FST among saline-treated mice, but had no effect in ACTH-treated mice (Fig. 2).

3.2. Effects of GLP-2 on immobility time in the FST

To investigate the effects of GLP-2 on ACTH-treated mice, we performed the FST after GLP-2 treatment (6 days, i.c.v.). Administration of GLP-2 (1.5–6 μg/mouse, i.c.v.) once a day for two days had no effect on immobility time (Fig. 3A). On the other hand, 6 days' treatment with GLP-2 significantly decreased immobility time in a dose-dependent manner (3 μg/mouse, Fig. 3B). Continuous administration (6 days) of GLP-2 induced also in the mice without ACTH. We previously reported that GLP-2 induced antidepressant-like effects on the 2nd day of the treatment [7]. Thus, the antidepressant-like effects were not weakened by continuous treatment.

3.3. Effects of imipramine or GLP-2 on serum corticosterone levels in ACTH treated mice

To investigate the effects of imipramine or GLP-2 on the stress response in ACTH-treated mice, we assayed serum corticosterone concentrations before and immediately after the FST. As shown